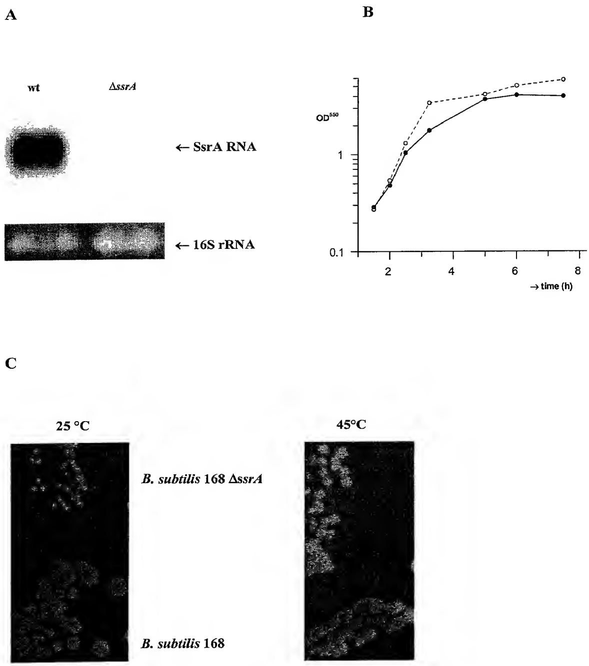


FIG. 1. A. Northern blot of total RNA of *B. subtilis* 168 and *B. subtilis* 168 Δ*ssrA*, hybridized with an *ssrA* specific probe. At the bottom: the level of 16S RNA in both RNA samples. B. Growth curves of *B. subtilis* 168 (---o---) and *B. subtilis* 168 Δ*ssrA* (—•—) at 37 °C in TSB medium. C. Growth of *B. subtilis* 168 and *B. subtilis* 168 Δ*ssrA* on HI-agar plates at 25 °C or 45 °C.

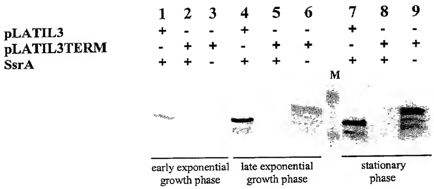


FIG. 2. hIL-3 expressed from an mRNA without a stop codon (pLATIL3TERM), accumulates in the medium of *B. subtilis* lacking SsrA (lanes 3, 6, 9), but not in cells containing functional SsrA (lanes 2, 5, 8). At three different growth stages, samples were collected from cultures of *B. subtilis* 168 (pLATIL3) [lanes 1, 4, 7], *B. subtilis* 168 (pLATIL3TERM) [lanes 2, 5, 8], and *B. subtilis* 168 Δ*ssrA* (pLATIL3TERM) [lane 3, 6, 9]. After centrifugation, the proteins in the culture supernatants were concentrated by TCA precipitation and analyzed by SDS-PAGE and Western blotting with anti-hIL-3 antibodies. The amount of total extracellular protein of *B. subtilis* 168 (pLATIL3) that was applied to the gel [lanes 1, 4, 7] was 10 times less then that of *B. subtilis* 168 (pLATIL3TERM) [lanes 2, 5, 8] or *B. subtilis* 168 Δ*ssrA* (pLATIL3TERM) [lanes 3, 6, 9]. M indicates a lane with a prestained protein ladder; the molecular weight of the upper band corresponds to 20 kDa, that of the lower band to15 kDa.

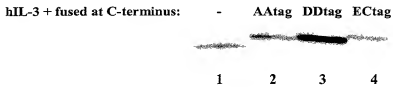
FIG. 3. Stability of hIL-3 variants with different C-terminal tags.

(A). Western blot analysis of hIL-3 protein variants produced by *B. subtilis* 168 transformed with plasmid pLATIL3 (lane 1), pLATIL3BStag (expression of hIL-3 with a C-terminal *B. subtilis* SsrA tag (AA-tag): hIL-3-AGKTNSFNQNVALAA; lane 2), pLATIL3DDtag (expression of hIL-3 with a DD-tag: hIL-3-AGKTNSFNQNVAL**DD**; lane 3), and pLATIL3ECtag (expression of hIL-3 with a C-terminal *E. coli* SsrA tag (EC-tag): hIL-3-AANDENYALAA; lane 4). Culture supernatants of cells entering the stationary phase were collected and analyzed by SDS-PAGE and Western blotting with anti-hIL-3 antibody.

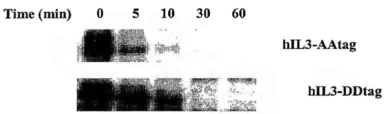
(B). Pulse-chase assays: Cells of *B. subtilis* 168 (pLATIL3BStag) and 168 (pLATIL3DDtag) were labeled with [^{35}S]-methionine for 1' prior to chase with excess non-radioactive methionine. Samples were withdrawn at the times indicated, centrifuged and the culture supernatants were analyzed by SDS-PAGE and fluorography.

(C). The amounts of hIL-3-AAtag and hIL3-DDtag in (B) were quantified by determination of the radioactivity in the dried gel using a PhosphorImager (Molecular Dynamics) and plotted.

A



B



C